

Sug 2
2 A method for treatment of Pythiosis in human
patients having the disease which comprises:

D1
3 (a) providing a vaccine containing a mixture
4 of proteins of *Pythium insidiosum* in a sterile aqueous
5 solution, wherein the mixture of proteins is (1) of
6 mixed intracellular proteins removed from disrupted
7 cells of the *Pythium insidiosum* grown in a culture
8 medium and (2) of mixed extracellular proteins from the
9 culture medium for growing the *Pythium insidiosum*[;]
10 wherein the mixed intracellular proteins and the mixed
11 extracellular proteins in water have been dialyzed to
12 remove low molecular weight components less than 10,000
13 MW; and

14 (b) vaccinating the patient with the vaccine.

1 *Sub 13* A method for the treatment of Pythiosis in a
2 mammal having the disease which comprises:

3 (a) providing an injectable vaccine derived
4 from growing cells of *Pythium insidiosum* in a culture
5 medium which comprises in a sterile aqueous solution in
6 admixture:

7 (1) mixed intracellular proteins removed from
8 disrupted cells of the *Pythium insidiosum*; and

9 (2) mixed extracellular proteins removed from
10 a supernatant from growing the cells of the *Pythium*
11 *insidiosum*;

12 wherein the mixed intracellular proteins and the mixed
13 extracellular proteins in water have been dialyzed to
14 remove low molecular weight components less than 10,000
15 MW to product the vaccine; and

16 (b) vaccinating the mammal with the vaccine.

-19- (Twice Amended)

Sub 73
cont

1 The method of Claim 18 wherein the removed proteins
2 in the admixture have been provided by growing cells of
3 the *Pythium insidiosum* in the culture medium, then
4 killing the cells, then separating the killed cells from
5 the culture medium to produce a first supernatant
6 containing the mixed extracellular proteins and then
7 disrupting the killed cells in sterile water to provide
8 the mixed intracellular proteins in a second supernatant
9 and separating [removing] the mixed intracellular
10 proteins from the disrupted cells and removing the mixed
11 extracellular proteins from the first and second
12 supernatants [supernatant].

-24- (Twice Amended)

Sub 74

1 The method of Claim 19 wherein the mixed
2 intracellular protein in the second supernatant are
3 separated from the disrupted cells [are removed from the
4 culture medium for the cells] by centrifugation and
5 removal of the disrupted cells [to provide the mixed
6 intracellular proteins in the second supernatant].